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Swedish wheat in the changing climate:

Screening for stable spring wheat genotypes from 2017 and 2018 with focus on protein quality for bread-making

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Svenskt vete i ett varierande klimat: Screening av vårvetegenotyper från 2017 och 2018 med fokus på proteinkvalité för brödtillverkning

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Abstract

In recent years, wheat production and protein quality in wheat have been affected by climatic changes in Sweden and in many other parts of the world. Protein quality of wheat, determining the bread-making quality of flour, is a parameter sensitive to climate fluctuations and its stability has not been studied so far. In order to have good and stable bread-making quality characteristics under varying climate, it is important to screen for climate resilient wheat varieties, which could later be used in breeding of climate stable wheat. Therefore, the aim of this thesis was to study the effects of temperature and precipitation on the wheat gluten protein characteristics and to screen for climate stable wheat genotypes. Thirty spring wheat genotypes were grown in contrasting climates in 2017 and 2018. The wheat gluten protein parameters were studied using size exclusion high-performance liquid chromatography (SE-HPLC). Grain structural morphology was evaluated by light microscopy (LM). The results showed that contrasting climate conditions significantly affected most of the gluten protein parameters (TOTE, TOTU, %LUPP, %UPP and %LUMP). Higher amounts of polymeric gluten proteins were found in plants grown during the hot-dry season of 2018 compared to the cold-wet growing season of 2017. Four genotypes, Happy, 19, 22 and 28, had a higher percentage (>40%) of total unextractable polymeric protein (%UPP), which differed less than 5% between years (2017 and 2018). These genotypes were considered as having a stable bread-making quality. Structural morphology analysis of the wheat grain indicated the presence of gluten protein and starch distribution, as well as a weak inner structure of the grain components which limited paraffin embedding of the grain. Consequently, the tested LM methodology requires further development and improvement. This study concludes that some of the genotypes (Happy, 19, 22 and 28) might have the potential to withstand heat and drought and still provide attractive bread-making characteristics. However, further tests (SE-HPLC analysis, rheomix and baking) of these genotypes need to be done, preferably during several years, in order to analyse their bread-making stability.

Sammanfattning

Under de senaste åren har veteproduktionen och proteinkvaliteten i vete påverkats av klimatförändringar i Sverige och i många andra delar av världen. Proteinkvaliteten i vete bestämmer mjölets kvalitet och bakegenskaper, men är känslig för klimatfluktuationer. Stabiliteten för denna parameter har inte studerats hittills. För att få en bra och stabil proteinkvalitet för brödbak vid varierande klimat, är det viktigt att identifiera klimatresistenta vetesorter, som också kan användas vid förädling av klimatstabilt vete. Syftet med denna studie var därför att studera effekten av temperatur och nederbörd på veteglutens egenskaper, samt att screena för klimatstabila vetegenotyper. Trettio genotyper av vårvete odlades i kontrasterande klimat under 2017 och 2018. Olika parametrar för vetegluten studerades med användning av size exclusion high-performance liquid chromatography (SE-HPLC). Vetekornens strukturella morfologi utvärderades med ljusmikroskopi (LM). Resultaten visade att kontrasterande klimatförhållanden signifikant påverkade de flesta av parametrarna för glutenprotein (TOTE, TOTU, %LUPP, %UPP och %LUMP). Högre mängder av polymera glutenproteiner hittades för de plantor som odlats under den varma och torra växtsäsongen 2018 jämfört med den kalla och våta växtsäsongen 2017. Fyra genotyper, Happy, 19, 22 och 28, hade en högre andel (>40%) icke-extraherbara polymeriska proteiner (%UPP), men värdena skilde mindre än 5% mellan åren 2017 och 2018. Dessa genotyper identifierades som stabila vad gäller kvalitet för brödbak. Analys av den strukturella morfologin hos vetekornet visade fördelningen av glutenprotein och stärkelse, men den bräckliga inre strukturen begränsade möjligheten till paraffininbäddning av vetekornen. Följaktligen krävs vidareutveckling och förbättring av ljusmikroskopimetoden. Slutsatsen från denna studie är att några genotyper, Happy, 19, 22 och 28, verkar ha potential att motstå värme och torka och kan under dessa förhållande producera vete med goda bakegenskaper. Det krävs dock ytterligare tester (SE-HPLC-analys, rheomix och bakning) av dessa genotyper samt odling under flera år, för att kunna analysera hur stabila deras bakegenskaper är.

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Abbreviations

Abbreviations	Meaning
mln.	million
ha.	hectares
t.	tonnes
HMW-GS	High Molecular Weight-Glutenin Subunits
LMW-GS	Low Molecular Weight- Glutenin Subunits
SE-HPLC	Size Exclusion High-Performance Liquid Chromatography
LM	Light Microscopy
SDS	Sodium Dodecyl Sulfate
MP	Monomeric Proteins
PP	Polymeric Proteins
eLPP	extractable Large Polymeric Proteins
eSPP	extractable Small Polymeric Proteins
eLMP	extractable Large Monomeric Proteins
eSMP	extractable Small Monomeric Proteins
uLPP	unextractable Large Polymeric Proteins
uSPP	unextractable Small Polymeric Proteins
uLMP	unextractable Large Monomeric Proteins
uSMP	unextractable Small Monomeric Proteins
TOTE	Total SDS-extractable proteins
TOTU	Total SDS-unextractable proteins
Mon/Pol	Ratio between monomeric and polymeric proteins
%LUPP	Percentage of large unextractable polymeric protein of total large polymeric protein
%UPP	Percentage of total unextractable polymeric protein of total polymeric protein
%LUMP	Percentage of large unextractable monomeric protein of total large monomeric protein
PFA	Paraformaldehyde
GA	Glutaraldehyde

1. Introduction

Wheat (*Triticum aestivum* L.) was one of the first domesticated food crops and has been cultivated for over 10 000 years (Husenov 2018). It is the third most important and cultivated food crop in the world (Röös et al. 2011; Land Lantbruk 2019; FAO 2020). The total wheat grown area in the world was about 219 million hectares (mln. ha.) in 2017 and the total global yield average over the years 2015 – 2017 was about 750 million tonnes (mln. t.) (Royo et al. 2017; FAO 2018; Husenov 2018). Wheat accounts for about 18% and 20% of the world's carbohydrate and protein consumption, respectively (Royo et al. 2017). In Sweden, around 15% of the arable land is used for wheat production (Land Lantbruk 2019). In 2017, the yield was 3.3 mln. t. from 471 000 ha. (Lyddon 2018). Most of the Swedish wheat is processed into flour, which is used either for food such as bread, pasta, breakfast cereals or for animal feed (Röös et al. 2011; Kuktaite 2004). In addition, wheat that is of non-food/feed grade can also be used for biofuel, as well as for production of bio-plastic materials of various types (Muneer et al. 2015 and 2016; Kuktaite et al. 2016; Andrade et al. 2018). For food purposes, quality and quantity of wheat storage proteins (gluten) are the major determinants of the end-use quality of wheat products such as bread, pasta, cookies etc. (Sharma et al. 2020). For food security reasons, different wheat breeding programs focusing not only on development of wheat varieties with improved gluten quality, but also on climate stability from year to year, are of high importance (Kiszonas & Morris 2018). This focus is becoming increasingly urgent, since climate seems more unpredictable and extreme, which drastically affects wheat production and quality on local and global levels (Kole et al. 2015).

1.1 Wheat protein and bread-making quality

Wheat grain contains approximately 65-75% starch, 10-12% proteins, 2-3% non-starch polysaccharides, 1-2% lipids and 14% water (Goesaert et al. 2005; Kuktaite 2004). Wheat gluten proteins are the main components determining the quality of wheat-based food products. Based on the protein solubility, wheat proteins can be divided into two types, gluten and non-gluten proteins (Figure 1). Approximately 85% is gluten protein and approx. 15% non-gluten protein. The gluten proteins are located in the endosperm of mature grains and are functioning as storage proteins, whereas the non-gluten proteins occur mostly in the outer layers of the wheat kernel and in minor quantities in the endosperm (Goesaert et al. 2005). Gluten proteins, in the presence of water and mixing energy, convert wheat flour into a dough with properties that are favourable

for bread-making (Husenov 2018). Thus, for a good bread-making quality, gluten protein properties are of the highest importance (Goesaert et al. 2005).

Wheat gluten proteins can be divided into two different types, gliadins and glutenins, according to their functionality. The proportions of gliadins and glutenins in gluten are approximately similar, around 45-55% (Figure 1). Gliadins are monomeric, while glutenins are polymeric in their nature. Gliadins are divided into four groups named alfa-, beta-, gamma- and omega-gliadins. Glutenins are polymeric proteins that are mostly formed through disulfide bridges between the subunits. These subunits are high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) (Husenov 2018; Johansson et al. 2013; Hussain 2012). High-molecular-weight glutenins determine gluten strength of the dough and are known to have a higher impact on the bread-making quality than the LMW-GS (Zhao et al. 2020). There are three specific loci in the wheat chromosomes, named Glu-A1, Glu-B1 and Glu-D1, that control the structure of HMW-GS and these loci are located on the long arms of the chromosomes 1A, 1B and 1D (Husenov 2018; Malik 2009). The combinations of the subunits influence the baking quality of wheat flour as for example, for locus Glu-D1, a combination of subunits Dx5 and Dy10 gives a stronger dough compared to Dx2 and Dy12 (Pfluger 2019; Malik 2009). This is mostly because of an extra cysteine residue in subunit Dx5 compared to subunit Dx2 (Malik 2009; Blechl et al. 2007).

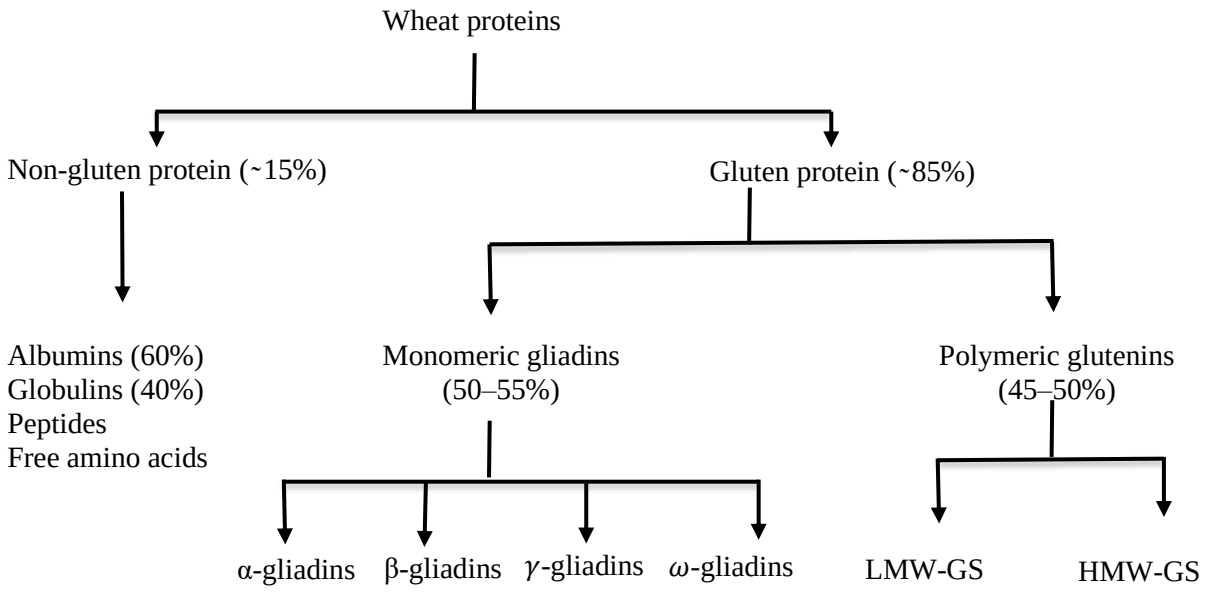


Figure 1: Classification of wheat protein according to Osborne solubility (Osborne 1907) with some modifications according to Goesaert et al. (2005) and Husenov (2018).

1.2 Climate change and factors affecting wheat protein and bread-making quality

Climate is changing rapidly due to global warming caused by the emission of different greenhouse gases and other factors. In the Scandinavian countries, the climate is expected to change more than the average worldwide (Andréasson et al. 2004). In Sweden, it is expected that the temperature will increase by around 0.5°C by 2050, and that the precipitation will fluctuate (SMHI 2019). For each degree rise in temperature, the global wheat production is estimated to be reduced by 6% (Akter & Islam 2017). Higher precipitation will increase water run-off, which will lead to a greater risk of leaching of nutrients and toxic substances (pesticides/herbicides) into rivers or ponds and decrease the availability of nutrients for wheat (Tsiouris et al. 2002). Optimum nutrient availability during the wheat grain development is very important in order to achieve the desired wheat grain quality characteristics. For some European countries, an increase of wheat harvest during diverse scenarios of climate change has been predicted by the researchers (Harrison & Butterfield 1996; Eckersten 2001; Kapur et al. 2019).

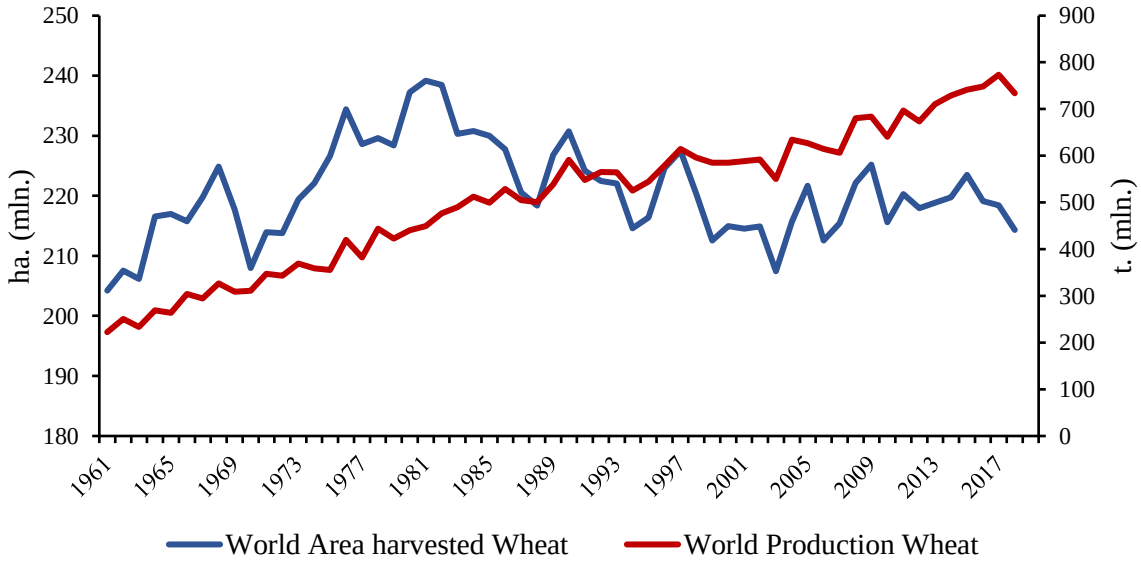
Gluten protein concentration, composition and accumulation in the grain during the grain development and at maturity, determine the quality of wheat flour and final end-use as for example, the bread-baking performance (Malik et al. 2013). These end-use qualities are influenced by different factors such as genotype (G) and growing environment (E), as well as by the interaction of these two (GxE). Environmental factors such as temperature, precipitation, fertilizer and level of CO_2 etc. have complex and interacting effects on the development of wheat grains and directly have impact on the yield and quality of wheat flour (Groos 2003; Altenbach. 2012; DaMatta et al. 2010). Temperature influences the gluten protein concentration and all the types of gluten proteins during the grain development period and at maturity (Johansson et al. 2005). High temperature and drought have been shown to shorten the duration of wheat grain development, while high temperature during the post-anthesis period of the crop can cause a reduction in grain size, grain setting and milling yield (Nuttall et al. 2017; Spiertz et al. 2006). Protein content in the grain was found to be lower for grains grown in lower temperatures (*e.g.*, 13°C), and to increase with higher temperatures (*e.g.* 20°C) (Koga et al. 2015 and 2016). Less protein in the grain grown at lower temperatures seems to be due to higher starch accumulation (Altenbach et al. 2003). In regard to wheat flour quality, a correlation between dough strength and an increase in protein concentration, and a high temperature during grain growing period (up to 30°C) has been found (Randall & Moss 1990; Johansson et al. 2005). Temperature and precipitation during wheat grain development are two important climatic factors that cause more

than 50% of the variation in yield and these factors may vary a lot between different years (Wiik & Ewaldz 2009; Erekul 2012). Precipitation is a major limiting factor for growing wheat, especially in semiarid and arid regions with rain-fed cropping systems (Guo et al. 2012). A higher precipitation promotes mould-damage of the wheat and pre-harvest sprouting, which further contributes to lower yield (Nielsen et al. 1984; Wiik & Ewaldz 2009; Li et al. 2013). On the other hand, too little precipitation during the wheat growing period leads to drought stress, which reduces yield but increases the amount of polymeric protein and gluten strength, thus improving bread volume and quality (Alghabari et al. 2015; Guzmán et al. 2016; Hernández-Espinosa et al. 2018; Li et al. 2013; Magallanes-Lopez et al. 2017).

1.3 Breeding for climate stable wheat

Plant breeding has been going on for many years from the time when local landraces were domesticated up to the development of modern wheat cultivars (Husenov 2018). This has been done to produce genotypes that provide high yields for a specific location and climate. Over the past 60 years, the yield of wheat genotypes has increased almost constantly throughout the world, even though the global cultivation area began to decrease about 30 years ago (Faostat 2019) (Figure 2A). For Sweden, especially during the last years, both the harvested area and the yield of wheat have increased. In 2017, the harvested area reached almost 500 thousand ha. and the yield was around 3.3 mln. t. (Figure 2B).

(A)



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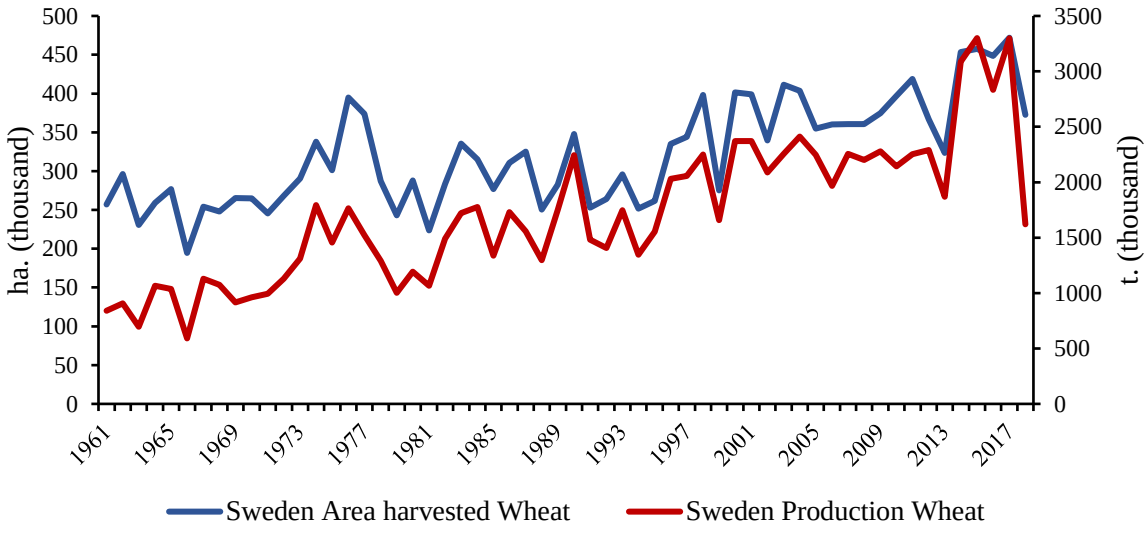


Figure 2: Total production and area harvested of wheat in (A) the World and (B) Sweden (Faostat 2019).

The goals of wheat breeding have always been to get high yields, as well as to have disease and pest resistant crops (Royo et al. 2017). The current focus of wheat breeders is not only the production of high yielding wheat varieties that are more environmentally friendly, e.g. are fertilizer efficient and disease resistant, but also the delivery of stable quality flour for bread-making in a changing climate (Raza et al. 2019). Varietal adaptation to a varying climate is very important because of the great impact of the climate change on agricultural practices and cultivation of wheat. New wheat varieties need to have a high climate adaptation in order to handle climate fluctuations such as excessive rain, drought and shifting temperatures (Raza et al. 2019).

For breeding and genotype development of bread wheat, the traditional and currently available screening methods for bread-making quality at early breeding stages are time consuming, rather expensive and laborious, and sometimes impossible to perform due to a small amount of seeds. In the wheat breeding process, there are thousands of breeding lines to screen for diverse quality parameters including bread-making characteristics. New faster methods, especially at early breeding stages, that are economically viable compared to the currently used in the quality labs are needed (Mwadzingeni et al. 2016; Gupta 1999).

1.4 Objectives

The specific objectives of this thesis are:

A) To study the effect of climate variation (temperature and precipitation) on different wheat gluten protein parameters (polymeric and monomeric glutenins) in 30 Swedish spring wheat varieties and breeding lines grown in 2017 and 2018 in Svalöv, Sweden by size-exclusion high performance liquid chromatography (SE-HPLC).

B) To evaluate climate impact (temperature and precipitation) on the wheat grain microstructure and the grain components morphology of the Swedish wheat genotypes by light microscopy (LM).

C) To identify genotypes that showed stable wheat gluten protein parameters profile during 2017 and 2018.

The focus of this study was to contribute with new knowledge towards the production of local Swedish spring wheat varieties with attractive bread-making quality characteristics that would be able to better tackle future climate fluctuations. Size exclusion high-performance liquid chromatography (SE-HPLC) was innovatively used to evaluate gluten protein quality stability, while light microscopy (LM) was used to map the grain microstructure and to study whether the distribution of protein and starch varies depend on diverse climatic conditions.

2. Materials and methods

2.1 Cultivation and growing conditions of the wheat used in this study

Thirty spring wheat cultivars/breeding lines grown during 2017 and 2018 (in total 60 samples) obtained from Lantmännen were included in this study (Table 1). The samples from 2017 were designated as, “A” and the samples from 2018 were designated as “B”, respectively.

Table 1: Name of the studied spring wheat genotypes.

Name of genotype	Sample nr.
Diskett	1
Sonett	2
Flippen	3
Happy	4
Quarna	5
Rogue	6
Mirakel	7
§	8
Bumble	9
Caress	10
Levels	11
§	12-30

Note: Names of the genotype marked with § are not authorized for publishing.

The genetic composition of the 30 genotypes for HMW-GS controlled by the loci Glu-A1 and Glu-D1 is shown in Table 2 (info provided by Lantmännen). The genotypes had the following combinations: 2*, 5+10; 1, 5+10; 2*, 1, 5+10 and 2*, 1, 2+12. Among the 30 tested genotypes the most frequent combination was 2*,1, 5+10, present in 15 breeding lines. There were a few that we did not have the genotype combination for.

Table 2: Genetical composition of glutenin subunits controlled by loci Glu-A1 and Glu-D1 for the studied wheat breeding lines.

Sample nr.	*Glu-A1*	*Glu-D1*
1-3	2*	5+10
4	1	5+10
5	-	-
6	2*, 1	5+10
7	-	-
8	-	-
9	1	5+10
10	2*	5+10

11-12	2*, 1	5+10
13-14	2*, 1	2+12
15-24	2*, 1	5+10
25	-	-
26	2*, 1	5+10
27	-	5+10
28	-	-
29	2*, 1	2+12
30	2*, 1	5+10

Note: No information of genotype combination is marked with -.

The spring wheat genotypes in this experiment were sowed on 25th of April in 2017 and on 20th of April in 2018. The harvest dates of the material were the 25th of September in 2017 and the 10th of August in 2018. Climatic conditions such as the average lowest and highest temperature and precipitation during the cultivation period of 2017 and 2018 are shown in Table 3.

Table 3: The climatic conditions (average lowest and highest temperatures, and total precipitation) during the growing periods of the studied wheat breeding lines in 2017 and 2018. Data recorded at the weather station at Lantmännen, Svalöv (http://www.ffe.slu.se/lm/LMHome.cfm?LMSUB=1).

Month	**Lowest Temperature (°C)**		**Highest Temperature (°C)**		**Total precipitation (mm)**	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**
April	0.4	6.1	10.1	15.6	3.8	18.6
May	7.1	9.8	17.0	21.8	21.6	33.6
June	10.9	11.6	18.9	22.9	88.6	19.2
July	11.4	13.6	19.6	26.0	78.0	3.6
August	11.6	16.0	20.7	26.8	110.2	13.4
September	9.7	-	16.5	-	96.0	-

2.2 Size exclusion high-performance liquid chromatography (SE-HPLC)

SE-HPLC method was used to separate the gluten protein components according to their sizes. For SE-HPLC analysis, grain from 30 wheat breeding lines from each of the years 2017 and

2018 (in total 60 samples) were grinded into flour using a Retsch Ultra Centrifugal Mill ZM 200. Flour samples were then freeze-dried (Cool safe Pro, LaboGene A/S, Denmark) for 3 days in order to remove humidity from the samples. Sixteen and a half mg of flour were weighted in triplicate and used for extracting proteins for SE-HPLC analysis. Protein concentration of wheat flour samples were measured using Near-Infrared Transmission (NIT) spectroscopy by a Foss Infratec 1241 Grain Analyser, FOSS Analytical A/S, Denmark.

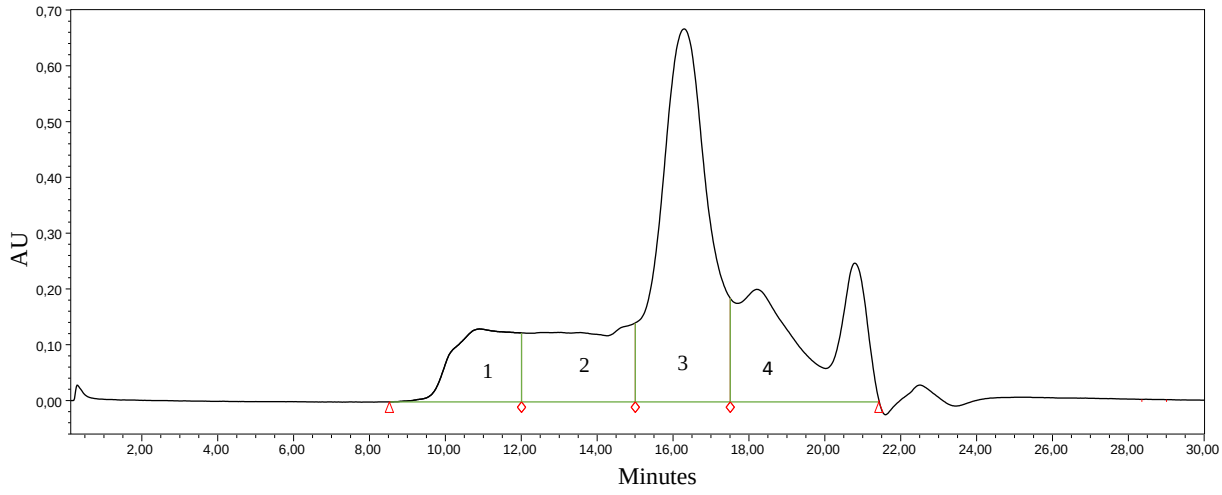
2.2.1 Protein extraction for SE-HPLC

Wheat gluten protein extractions of SDS-extractable protein (extraction 1) and SDS-unextractable protein (extraction 2) were done using two extraction steps according to Gupta et al. (1993), with some modifications according to Kuktaite et al. (2016). For the gluten protein extraction from the flour, an extraction buffer containing 0.05 M NaH_2PO_4H, 0.5% SDS, pH 6.9 was used. For extraction 1, 1.4 ml of the extraction buffer was added to 16.5 mg of wheat flour, and the mixture was vortexed for 5 minutes at 2000 rpm. Then the samples were centrifuged at 10 000 rpm and the supernatant was carefully collected for further SE-HPLC analyses. For extraction 2, 1.4 ml of the extraction buffer was added to the remaining pellet collected after the first extraction. Then the sample was sonicated (Soniprep 150 untrasonic disintegrator, Sanyo, Japan) with 5 microns amplitude for 45 seconds and centrifuged at 10 000 rpm. The collected supernatant was used for SE-HPLC analysis. Typical SE-HPLC chromatograms of extraction 1 and extraction 2 are shown in Figure 3.

The protein amount and size distribution of the samples were analysed using a SE-HPLC system, (Waters 2690 Separation Module) connected to Waters 996 Photodiode Array Detector (Waters, USA). A size exclusion column (Biosep-SEC-S4000, Phenomenex, USA) was used for the protein separation, and a mobile phase consisting of 50% ACN and 0.1% TFA was used. For washing of the SE-HPLC column 80% methanol was used. Three-dimensional spectral data collection was performed at 190-220 nm, and proteins were detected at 210 nm UV absorbance. Twenty µL of the extracted protein was used for SE-HPLC analysis and run in the column for 30 minutes. Protein characterization in the chromatogram was done according to the method from Kuktaite et al. (2016). The polymeric protein (PP) interval was between 8.5 and 15 minutes, while the monomeric protein (MP) interval was between 15 and 21.5 minutes. The chromatogram was divided into four areas according to the molecular sizes such as, large polymeric proteins (LPP) representing gluten strength (interval 1; 8.5-12 minutes), small

polymeric proteins (SPP) (interval 2; 12-15 minutes), large monomeric proteins (LMP) (interval 3; 15-17.5 minutes) and small monomeric proteins (SMP) (interval 4; 17.5-21.5 minutes), are shown in Figure 3.

(A)



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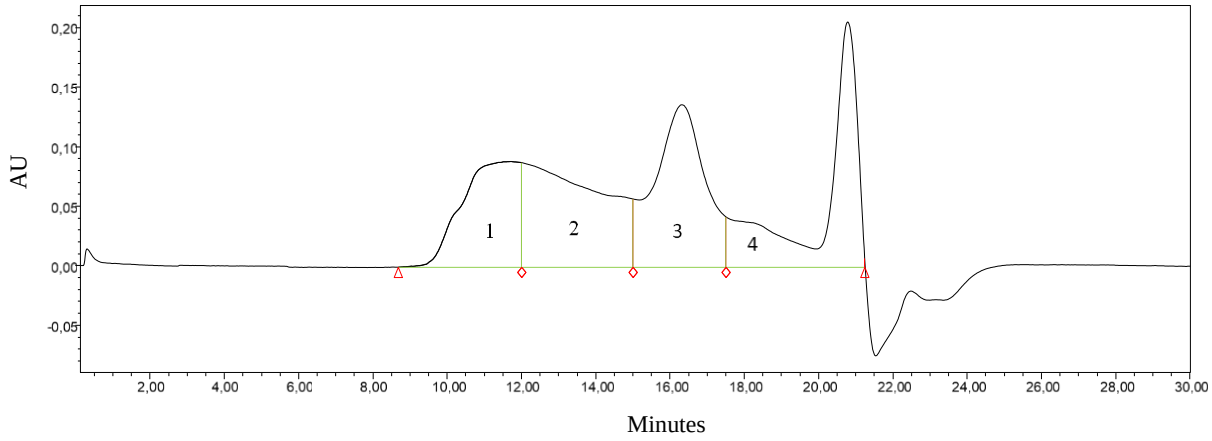


Figure 3: An example of chromatogram of (A) SDS-extractable, and (B) SDS un-extractable proteins for genotype 27 from 2017, where intervals 1-4 are referred to LPP, SPP, LMP and SMP fractions, respectively.

2.2.2 Calculations of protein parameters

The calculations of the polymeric and monomeric fractions were done according to the method developed by Gupta et al. (1993), with some modification by Kuktaite et al. (2016). The following protein parameters from the SE-HPLC separation were calculated: total SDS-extractable proteins (TOTE), total SDS-unextractable proteins (TOTU), percentage of large

unextractable polymeric protein of total large polymeric protein (%LUPP), percentage of total unextractable polymeric protein of total polymeric protein (%UPP), percentage of large unextractable monomeric protein of total large monomeric protein (%LUMP) and the ratio of monomers to polymers. The following formulas were used for calculation of the above-mentioned protein parameters:

TOTE = eLPP + eSPP + eLMP + eSMP

TOTU = uLPP + uSPP + uLMP +uSMP

%LUPP = uLPP/(uLPP + eLPP) × 100

%UPP = uLPP + uSPP/(eLPP + eSPP + uLPP + uSPP) × 100

%LUMP = uLMP/(uLMP + eLMP) ×100

$$\mathrm{Mon/Pol} = \frac{\text{monomer}}{\text{polymer}}$$

%UPP is referred to as one of the most important gluten protein fractions that correlates well with the gluten strength, as has been showed in several studies (Johansson et al. 2013; Malik et al. 2013). Mon/Pol is a parameter determining whether the flour is dominated by the monomeric protein fraction (responsible for gluten extensibility) or the polymeric protein fraction (responsible for gluten strength) (Tronsmo et al. 2002). Higher ratio of Mon/Pol results in a higher dough extensibility and a lower dough strength.

2.3 Light microscopy (LM)

Out of 30 genotypes, 4 were selected in order to study the effect of contrasting climate on the grain components morphology e.g. protein and starch distribution by LM. In order to evaluate the impact of climate variation (temperature and precipitation), on morphology and structure of wheat grain components such as protein and starch, and their distribution in the grain, the selected breeding lines were studied using LM. Two sample preparation methods were tested in order to find the optimum concentrations of the cross-linker and preparation conditions.

The first sample preparation method was implemented by soaking halves of wheat grain (divided along the groove) in milli-Q H_2O for 4 hours or overnight (Jääskeläinen et al. 2013). Soaked grains were then exposed to 0.1% light green solution for 1 minute to stain the proteins (green/blue colour) and later to 50% lugol (iodine) for 1 minute in order to stain starch (dark

lilac/black). Different dyes (0.1% fast green and 0.1% acid fuchsin) and staining times (1.5, 2, 3 and 5 minutes) were tested for colouration of wheat proteins.

A different method for studying the grain morphology and protein distribution was tested and included paraffin fixation of the grain samples according to Gibson (2017) with some changes. Different concentrations of the fixation solutions paraformaldehyde (PFA) and glutaraldehyde (GA) were evaluated. Four different combinations of PFA and GA, a) 3% PFA + 0.25% GA, b) 4% PFA + 0.25% GA, c) 4% PFA + 1% GA and d) 4% PFA + 2.5% GA, were tested. From the tested combinations, a blend of 4% PFA + 2.5% GA was selected for further analyses. Four genotypes, Sonett, 19, 21 and 28 grown under 2017 and 2018 were selected for LM structural analyses.

Before the fixation, the grains were soaked in milli-Q H_2O for at least 4 hours and then cut transversely and longitudinally to determine if there were any difference in the component morphology. The cut grains were then transferred to of glass vials (10 ml) which were further used throughout 7 fixation and paraffin embedding steps. During fixation (step 1), a solution of PFA, GA and 0.1 M Na phosphate (pH 7.2) were added to the cut wheat grains in the glass vials and the seeds were soaked for 4 hours in room temperature; the procedure was repeated overnight at +4°C on a shaker. Later, the seeds were washed (step 2) for 10 minutes using 0.2 M Na-phosphate buffer (pH 7.2) and dehydrated (step 3) using different ethanol solutions (30%, 50%, 70%, 90% and 95%) for at least 30 minutes per dehydration step, followed by a final dehydration step using absolute ethanol, repeated twice and with washing overnight. The specimens were further infiltrated (step 4) by washing with a blend of ethanol and xylene (2:1 ratio followed by 1:2 ratio), and then by only xylene solution that was repeated twice, for 1 hour or overnight with each of the solutions-steps. Paraffin embedding was applied in four steps (during 1 hour, overnight, during 8 hours and overnight), casted (step 5) in a mould and cooled on ice. The embedded samples were cut (step 6) and sectioned into thin slices around 15-20 µm thick, and sections were collected on slides using a cooler (SuperFrost Plus, VWR, Germany) for staining. Before staining, the paraffin was removed using a number of solvents such as xylene, xylene + ethanol, ethanol (absolute ethanol, 95%, 90%, 70%, 50%, 30%) and milli-Q H_2O. Each step took 5 minutes except the first xylene washing that took 10 minutes. Staining (step 7) was applied on the glass cuvettes using 0.1% light green solution for protein staining, for about 1 minute. The cuvettes were rinsed with milli-Q H_2O and afterwards stained with 50% lugol (iodine, 1 minute) for starch staining and then rinsed with milli-Q H_2O prior to LM analyses. Structural analysis

was done using LM (Leica microsystem, Germany) and a digital camera (Leica DFC450) attached to the LM.

2.4 Statistics

The statistical analyses were done using statistical software R and Minitab 19. ANOVA was conducted in order to evaluate the variance in protein parameters of the studied wheat genotypes grown under 2017 and 2018. The dependent variables were genotype (G), year (Y) and the interaction between genotype and year (GxY). Principal component analysis (PCA) was done using Minitab 19 to assess the pattern of variation of gluten proteins in genotypes grown in different years (2017 and 2018).

3. Results

3.1 Protein concentrations

The total protein concentration of wheat flour samples measured by Near-Infrared Transmission (NIT) spectroscopy showed variation between genotypes grown in 2017 and in 2018, as presented in Figure 4. The protein concentration in 12 of the genotypes was found to be higher in 2017 compared to 2018, whereas Sonett, Mirakel, Levels, 17, 23, 24 and 28 had higher content in 2018. The genotypes Levels, 14, 16, 17, 19 and 26 showed the smallest difference (less than 1%) in the flour protein concentration between the years (Figure 4), while Sonett, Quarna and 23 showed more than 2% difference.

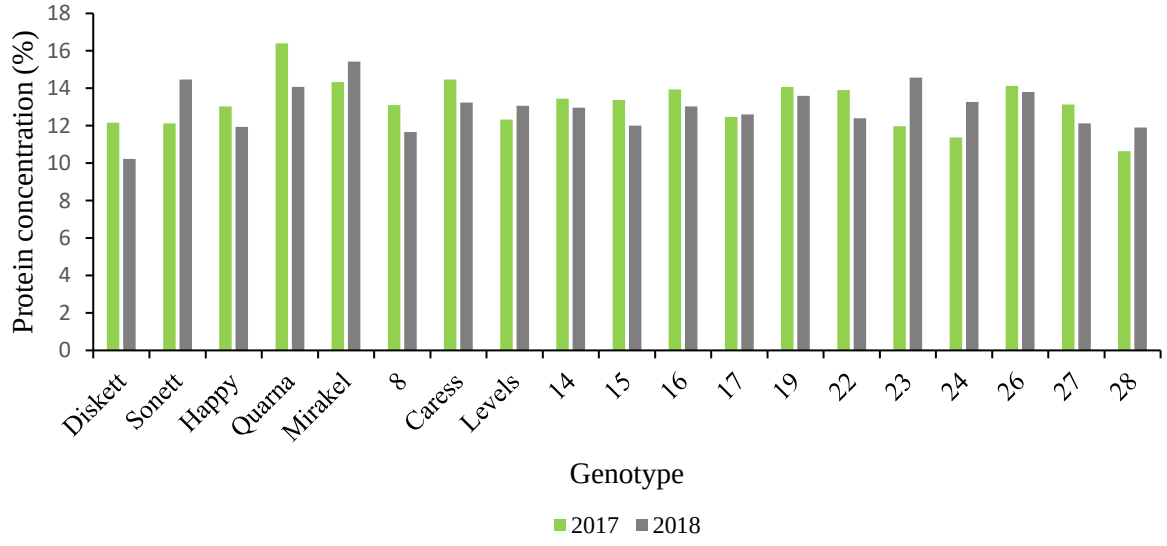


Figure 4: Protein concentration (%) of the flour samples of wheat genotypes grown in 2017 and 2018 as measured by NIT.

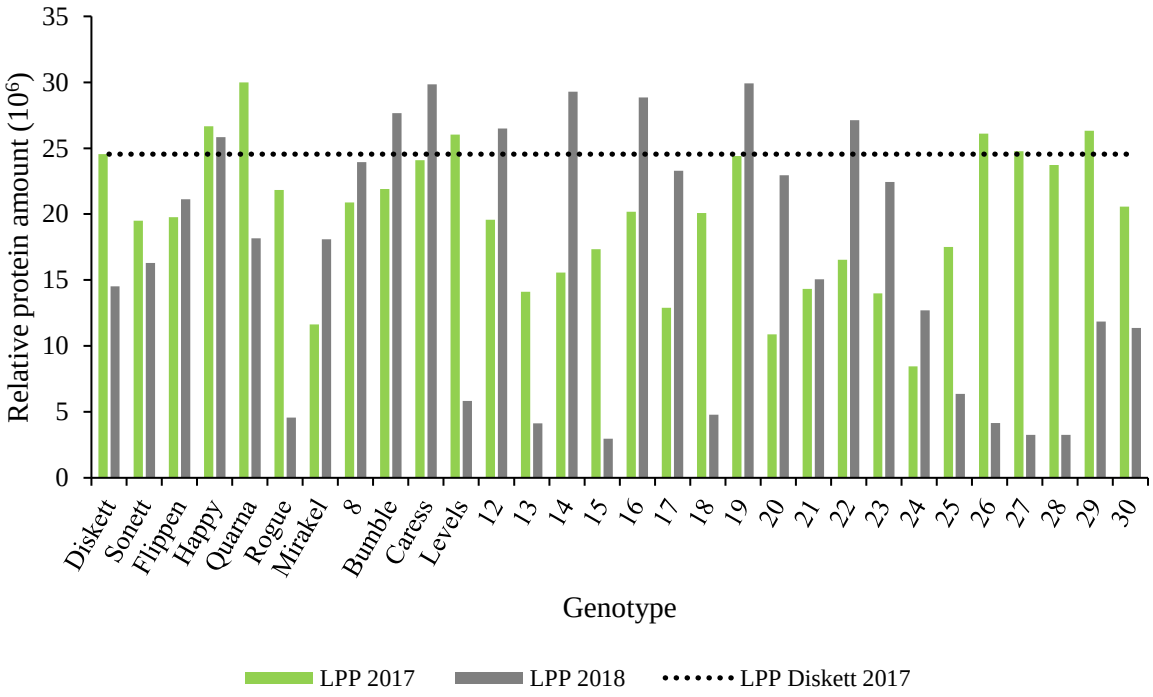
3.2 Gluten protein parameters studied by SE-HPLC

The SE-HPLC results for the total relative amounts of LPP, SPP, LMP and SMP for both SDS-extractable and SDS-unextractable proteins showed a large variation between years for the studied genotypes (Figure 5). The total amount of the large polymeric protein fraction (LPP), for the genotypes Happy, Quarna, Caress, Levels, 19, 26, 27, 28 and 29 grown in 2017 was found to be equal to or higher than Diskett from 2017, a standard industrial variety (Figure 5A; the dotted line indicates LPP for Diskett from 2017). For the genotypes grown in 2018, Happy, 8, Bumble, Caress, 12, 14, 16, 19 and 22 showed equal or higher LPP compared to Diskett from 2017. The total amounts of SPP for 23 genotypes in 2017 were found to be equal to or higher than Diskett from 2017 compared to 14 in 2018 (Figure 5B; the dotted line indicates SPP for Diskett from 2017). The most stable genotypes regarding relative amount of polymeric proteins (LPP and SPP) in 2017 and 2018 were Flippen, Happy and 21 for LPP, and Mirakel, 17, 20 and 30 for SPP.

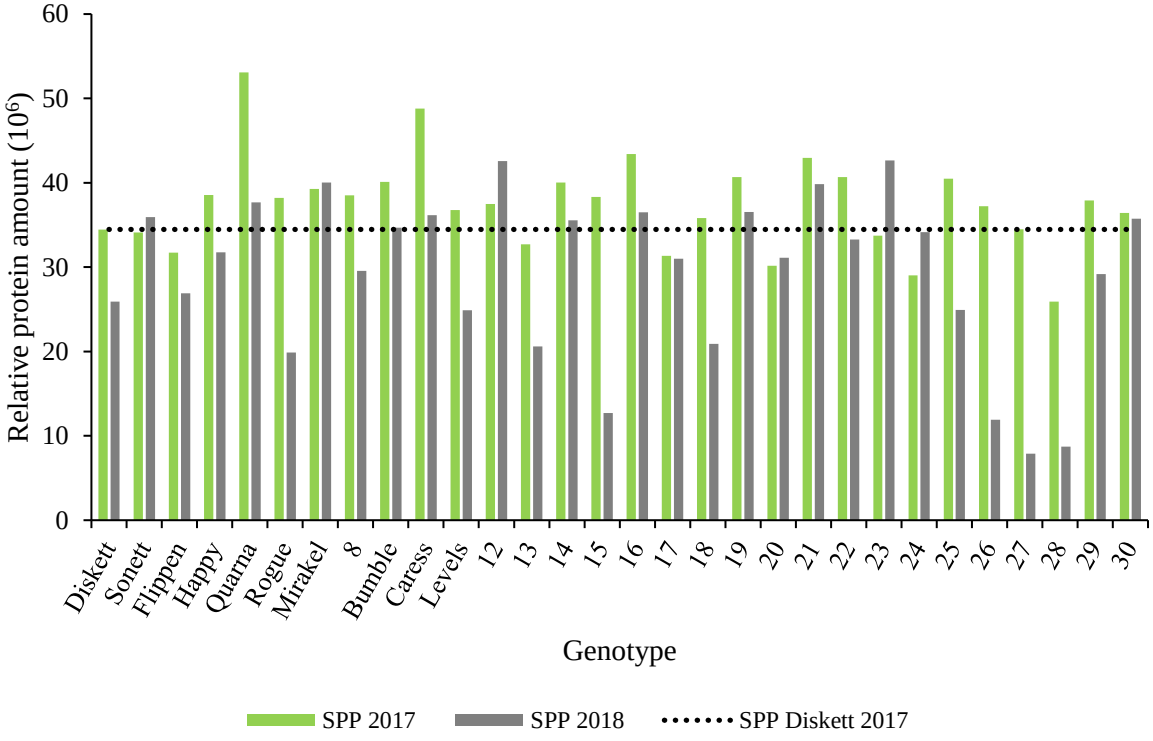
When compared between the years, heat and drought increased the amounts of large polymers in several of the genotypes (LPP), specially in the breeding lines 14, 16, 17, 20, 22 and 23 in 2018 compared to 2017, indicating positive drought-heat impact on gluten strength.

The relative amounts of LMP were found to be equal or higher for all the genotypes from 2017 (except Flippen and 28) when compared to Diskett from 2017 (Figure 5C; the dotted line indicates LMP for Diskett from 2017). For the genotypes grown in 2018, drought and heat increased the amount of LMP for most of the genotypes compared to Diskett from 2017, except Flippen, Happy, 8, 19, 20 and 28. The relative amounts of SMP in 2017 were found to be equal or higher for most of the genotypes, except Flippen and Happy, when compared to Diskett from 2017 (Figure 5D; the dotted line indicates SMP for Diskett from 2017). For 2018 most of the genotypes were found to be equal or higher, except Diskett and Flippen, when compared to Diskett from 2017. The most stable genotypes from 2017 and 2018 regarding relative amount of monomeric proteins (LMP and SMP) were Rouge, 17 and 25 for LMP, and Flippen and Mirakel for SMP (Figure 5).

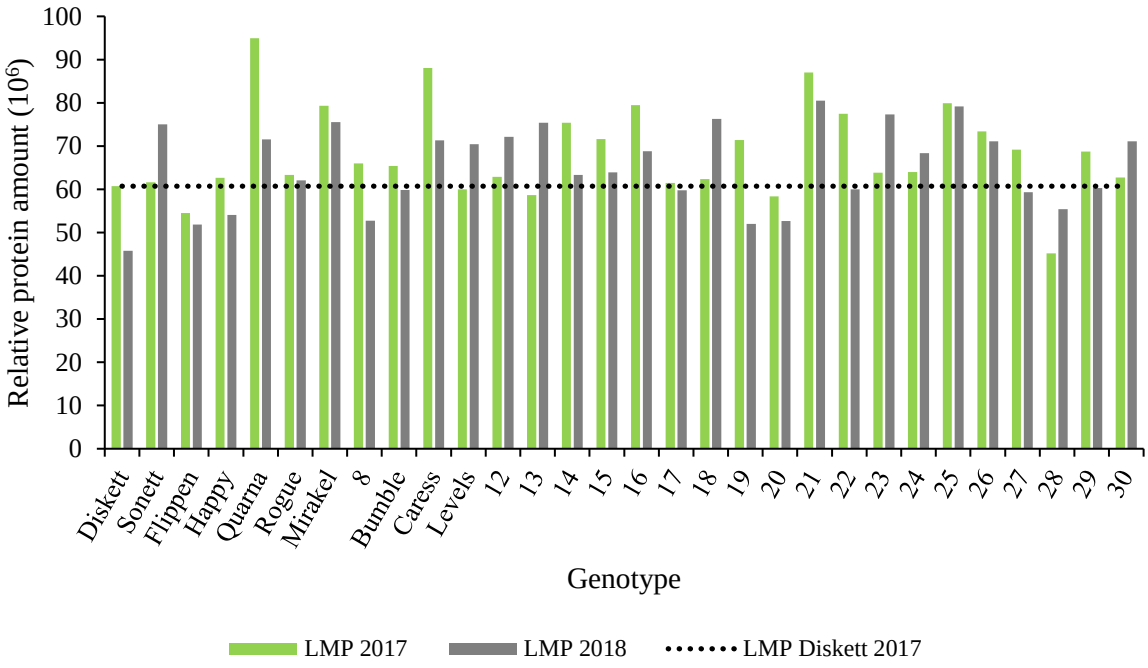
(A)


Relative protein amount (10^6)
35
30
25
20
15
10
5
0
Diskett
Sonett
Flippen
Happy
Quarna
Rogue
Mirakel
8
Bumble
Caress
Levels
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Genotype
LPP 2017
LPP 2018
LPP Diskett 2017

(B)


Relative protein amount (10^6)
60
50
40
30
20
10
0
Diskett
Sonett
Flippen
Happy
Quarna
Rogue
Mirakel
8
Bumble
Caress
Levels
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Genotype
SPP 2017
SPP 2018
SPP Diskett 2017

(C)



(D)

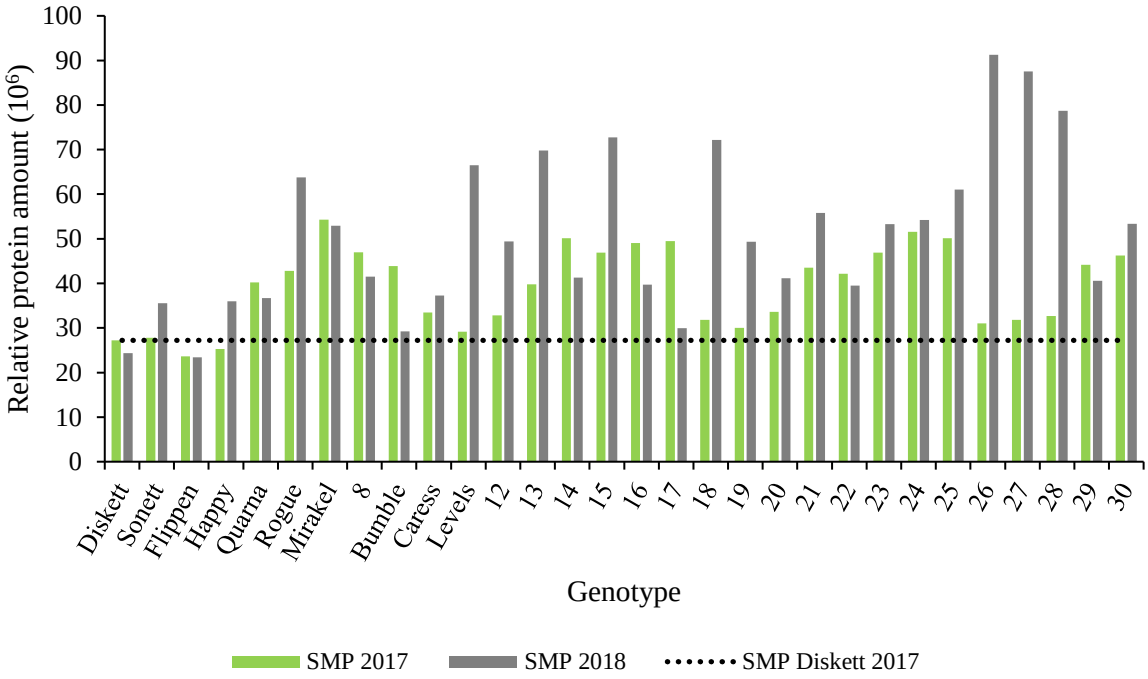


Figure 5: The relative amount of gluten protein calculated as, LPP (A), SPP (B), LMP (C) and SMP (D) (for both SDS-extractable proteins and SDS-unextractable proteins) for the studied wheat genotypes during both years 2017 and 2018. The dotted line indicates Diskett from 2017 for LPP, SPP, LMP and SMP in order to easily compare the samples.

Protein parameters TOTE, TOTU, %LUPP, %UPP and %LUMP calculated from SE-HPLC data were compared for the studied cultivars and wheat breeding lines grown in 2017 and 2018, and are shown in Table 4. The TOTE values were found to be higher in Diskett, Flippen, Happy, Quarna, Mirakel, 8, Bumble, Caress, and in breeding lines 14-17, 21, 22, 25 and 29 grown in 2017 compared to the same genotypes grown in 2018 (Table 4). The genotypes Sonett, Rogue, Levels, 12, 13, 18, 19, 20, 23, 24, 26, 27, 28 and 30 showed higher TOTE values when grown in 2018 compared to 2017, which indicates that heat and drought induced an increase in TOTE in approx. half of the genotypes (Table 4). The most stable genotypes, showing similar TOTE for both years, were Sonett, Rogue, Mirakel, 15, 19, 21, 22, 27 and 30 (≤5% difference between the years as stable genotypes).

TOTU and %LUPP parameters representing total unextractable proteins and percentage of large unextractable polymeric proteins, both playing an important role in bread-making performance, are shown in Table 4. Higher TOTU values, due to the heat and drought, that occurred in 2018 were found for Sonett, Flippen, Happy, Mirakel, Caress, Levels, 12, 16, 18, 20 and 21 (Table 4). The most stable genotypes in terms of TOTU were Flippen, Bumble, and the breeding lines 14, 16, 17, 19, 23, 25, 26, 28 and 30 which showed similar values, both of the studied years (≤5% difference between the years as stable genotypes). Regarding %LUPP, higher values were found for most of the genotypes grown in 2018 compared to 2017, except the breeding lines 13,15, 26-30 and the genotype such as, Rogue, (Table 4). The most stable genotypes for both years, were the breeding lines 13, 14 and 17 (≤5% difference between the years as stable genotypes).

%LUMP representing the large unextractable monomeric protein fraction in gluten. Higher values were found for the genotypes Sonett, Levels, 25-29 and lower values for genotypes 13, 20, 22, 23, 24 grown in 2018 compared to 2017 (Table 4). The stable genotypes were Rouge, Mirakel, 8, Caress and 16 (≤5% difference between the years as stable genotypes).

Table 4: Relative amounts of wheat gluten protein calculated as TOTE, TOTU, %LUPP and %LUMP for cultivars and breeding lines from 2017 and 2018.

Genotype	TOTE (10^6)		TOTU (10^6)		%LUPP		%LUMP	
	2017	2018	2017	2018	2017	2018	2017	2018
Diskett	111.4	80.4	35.6	30.2	30.9	39.4	16.9	20.4
Sonett	107.4	108.3	35.7	54.5	22.5	45.3	22.0	28.7
Flippen	84.2	76.2	45.4	47.1	43.7	52.3	29.0	31.8
Happy	100.3	91.4	52.9	56.3	49.0	54.0	24.2	27.5
Quarna	144.0	108.2	74.3	55.9	53.2	61.4	22.5	25.3
Rogue	106.9	112.1	59.3	38.2	52.1	49.0	25.4	24.9
Mirakel	119.7	115.2	64.8	71.3	49.8	69.3	28.8	29.3
8	116.7	97.0	55.6	50.7	51.1	59.5	22.5	21.5
Bumble	113.1	95.1	58.1	56.4	55.8	65.6	21.6	24.9
Caress	152.8	125.6	41.5	49.0	28.7	51.3	15.4	15.0
Levels	105.6	113.1	46.4	54.5	46.3	50.8	21.4	29.9
12	109.1	127.9	43.6	62.7	45.1	56.0	20.5	21.7
13	102.8	134.1	42.4	35.8	44.7	42.5	22.0	17.3
14	133.2	123.6	47.9	45.9	44.3	46.3	18.0	14.9
15	118.6	116.6	55.6	35.7	51.7	45.9	23.4	21.5
16	138.6	117.8	53.4	56.0	44.7	57.0	19.6	19.6
17	109.1	98.4	46.0	45.6	59.8	60.7	18.7	20.8
18	110.4	126.3	39.6	47.9	26.9	47.8	23.5	25.5
19	100.2	104.2	66.3	63.6	48.8	60.1	34.3	31.4
20	92.8	103.8	40.2	44.0	28.4	50.6	29.0	18.7
21	127.6	124.0	60.3	67.2	36.8	72.2	29.0	26.2
22	112.8	107.7	64.1	52.2	45.9	53.4	33.5	20.9
23	100.5	137.1	57.9	58.6	48.9	56.5	30.6	20.3
24	91.6	118.6	54.3	50.9	39.0	66.0	32.3	21.4
25	130.7	115.2	57.3	56.3	47.4	56.4	23.2	32.0
26	121.0	131.9	46.8	46.5	40.0	32.2	21.5	28.9
27	118.1	121.8	42.1	36.2	40.6	32.3	19.4	25.2
28	87.8	106.2	39.8	39.9	50.7	32.9	19.4	30.9
29	126.2	105.3	50.9	36.5	41.1	20.3	19.7	23.0
30	112.4	117.3	53.6	54.2	47.3	32.4	22.8	27.8

For the studied genotypes, %UPP varied between 30-58% (Figure 6). In this study, we chose to differentiate the genotypes having >40% of %UPP and ≤5% difference between the years as stable genotypes. The stable genotypes regarding %UPP were Happy, 19, 22 and 28 (Figure 6; marked with blue arrows). In general, higher %UPP was observed for most of the genotypes grown in 2018, compared to the 2017 harvest. As for the intermediate stable genotypes, they had a percentage of %UPP of >30% and a difference in %UPP of ≤11% between the years. The intermediate stable genotypes were Quarna, Rogue, Levels, 14, 15, 17, 23 and 24 (Figure 6; marked with red arrows).

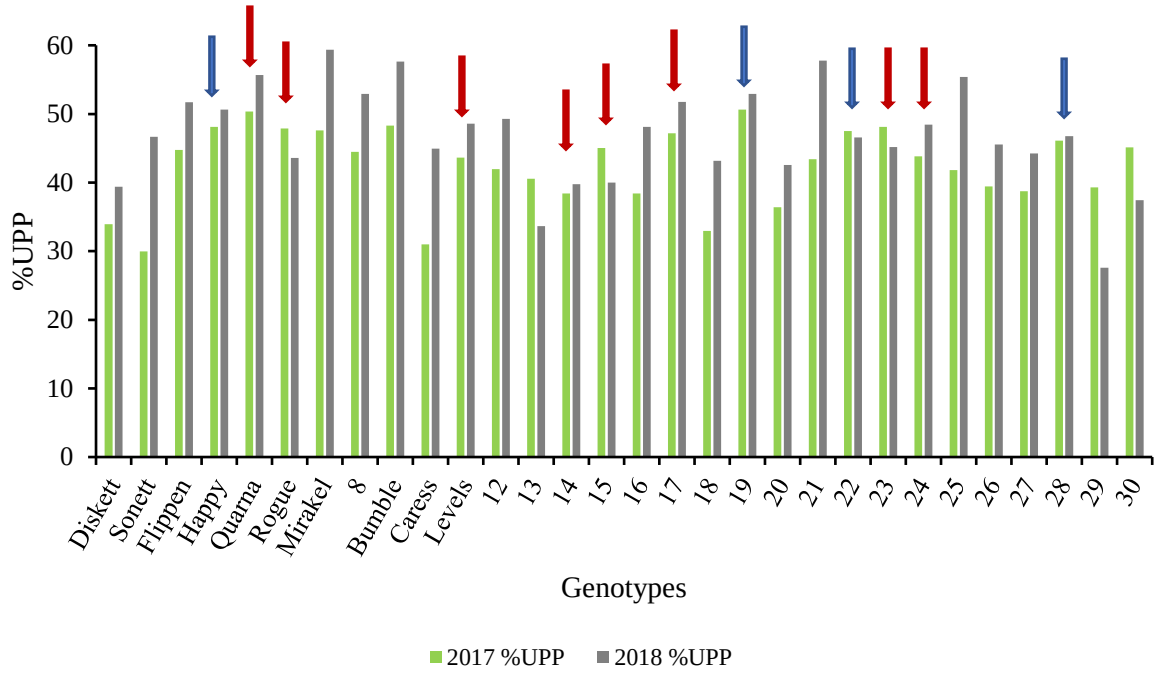


Figure 6: Percentage of UPP calculated from SE-HPLC data of 30 spring wheat genotypes grown in 2017 and 2018. Blue arrows are showing the most stable wheat genotypes that have a percentage above 40% and a difference between years lower than 5%. Intermediate stable genotypes are marked with red arrows.

The Mon/Pol ratio in this study was found that the value of Mon/Pol ratio was significantly higher in Rogue and Levels, and the breeding lines 13, 15, 18, 25-28 grown in 2018, compared to the same genotypes grown in 2017 (Figure 7). In these genotypes, a dominating amount of monomers was found. The rest of the studied genotypes were having either a small or none significant difference between the years (Figure 7).

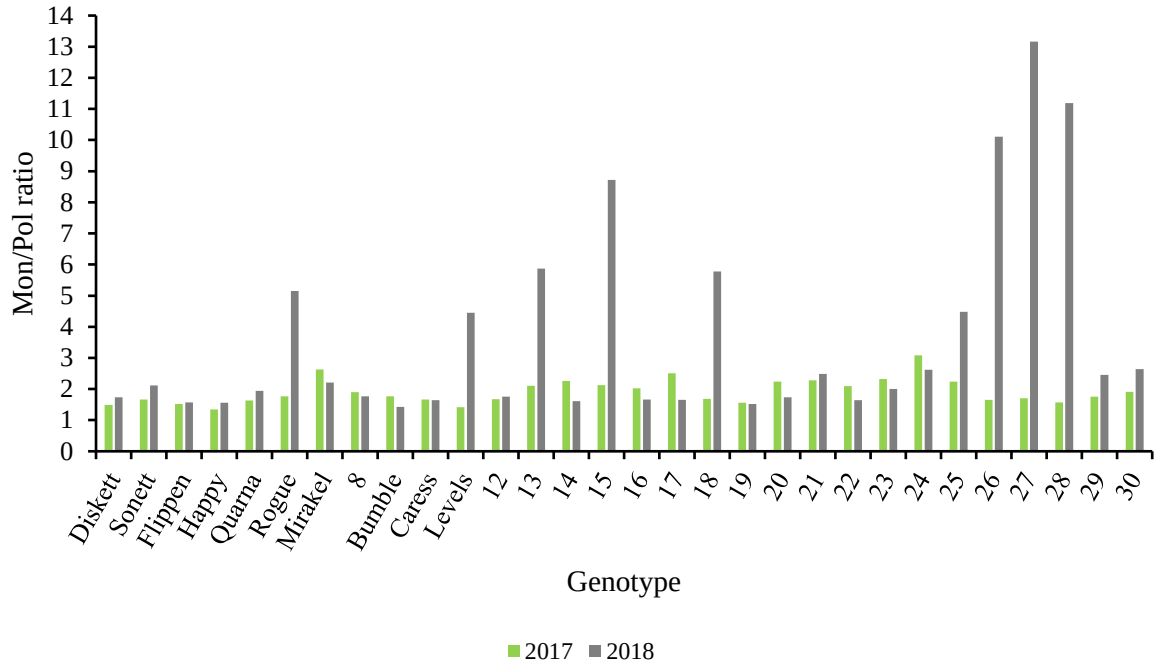


Figure 7: Mon/Pol ratio of 30 spring wheat genotypes grown in 2017 and 2018.

3.3 Impact of genotype (G), year (Y) and the interaction GxY on the protein parameters

Genotype (G), year (Y) and the interaction GxY was found to significantly influence nearly all the studied protein parameters, i.e. eLPP, eSPP, eLMP, eSMP, uSPP, uSMP, TOTU, %LUPP, %UPP and Mon/Pol (Table 5). The protein parameters uLPP, uLMP, TOTE and %LUMP were significantly influenced by G and GxY, and TOTE, at $p<0.05$, was significantly influenced by Y. No significant impact of Y on uLPP, uLMP and %LUMP was observed, indicating that these parameters were resistant to the contrasting climate in 2017 and 2018 (Table 5).

Table 5: The mean squares from ANOVA for relative amounts of SDS-extractable proteins and SDS-unextractable proteins for LPP, SPP, LMP, SMP, TOTE, TOTU, %LUPP, %UPP, %LUMP, Mon/Pol for 30 studied spring wheat genotypes (G), two growing years (Y) and the interaction of GxY.

	Genotype (G)	Year (Y)	Genotype:Year (GxY)	Residuals
Df	29	1	29	120
eLPP (10^{14})	0.47***	5.59***	0.43***	0.01
eSPP (10^{14})	0.80***	13.76***	0.54***	0.02
eLMP (10^{14})	2.86***	2.39***	1.13***	0.07
eSMP (10^{14})	3.21***	38.78***	3.00***	0.05
uLPP (10^{13})	6.45***	0.24	6.70***	0.12
uSPP (10^{14})	0.78***	3.09***	0.25***	0.01
uLMP (10^{13})	6.48***	0.86	3.87***	0.26
uSMP (10^{13})	1.38***	1.83***	1.88***	0.06
TOTE (10^{14})	8.24***	1.94*	4.05***	0.30
TOTU (10^{14})	4.17***	2.15***	1.15***	0.01
%LUPP (10^{3})	0.43***	2.21***	0.27***	0.02
%UPP (10^{2})	1.85***	8.98***	0.75***	0.09
%LUMP	77.72***	2.94	56.20***	5.71
Mon/Pol	13.24***	114.59***	14.25***	0.20

Asterisks indicate significance, p value at ***p<0.001, **p<0.01, *p<0.05.

3.4 PCA of the protein parameters

The eigenvectors and eigenvalues of a correlation matrix representing two principal components (PC) of the studied protein parameters eLPP, eSPP, eLMP, eSMP, uLPP, uSPP, uLMP, uSMP, TOTE, TOTU, %LUPP, %UPP, %LUMP and Mon/Pol are shown in Table 6. The first two components accounted for 61% of the total variability, of which PC1 and PC2 explained 34% and 27%, respectively (Figure 8). The distribution of the data from 2017 was more clustered compared to the values from 2018 (Figure 8).

Table 6: Eigenvectors for the two first principal components for eLPP, eSPP, eLMP, eSMP, uLPP, uSPP, uLMP, uSMP, TOTE, TOTU, %LUPP, %UPP, %LUMP and Mon/Pol.

Variable	PC1	PC2
eLPP	0.35	0.25
eSPP	0.35	0.14
eLMP	0.09	0.02
eSMP	-0.38	-0.14
uLPP	0.38	-0.10
uSPP	0.37	-0.25
uLMP	-0.05	-0.38
uSMP	-0.25	-0.26
TOTE	-0.03	0.01
TOTU	0.21	-0.43
%LUPP	0.17	-0.35
%UPP	0.10	-0.44
%LUMP	-0.08	-0.34
Mon/Pol	-0.42	-0.05

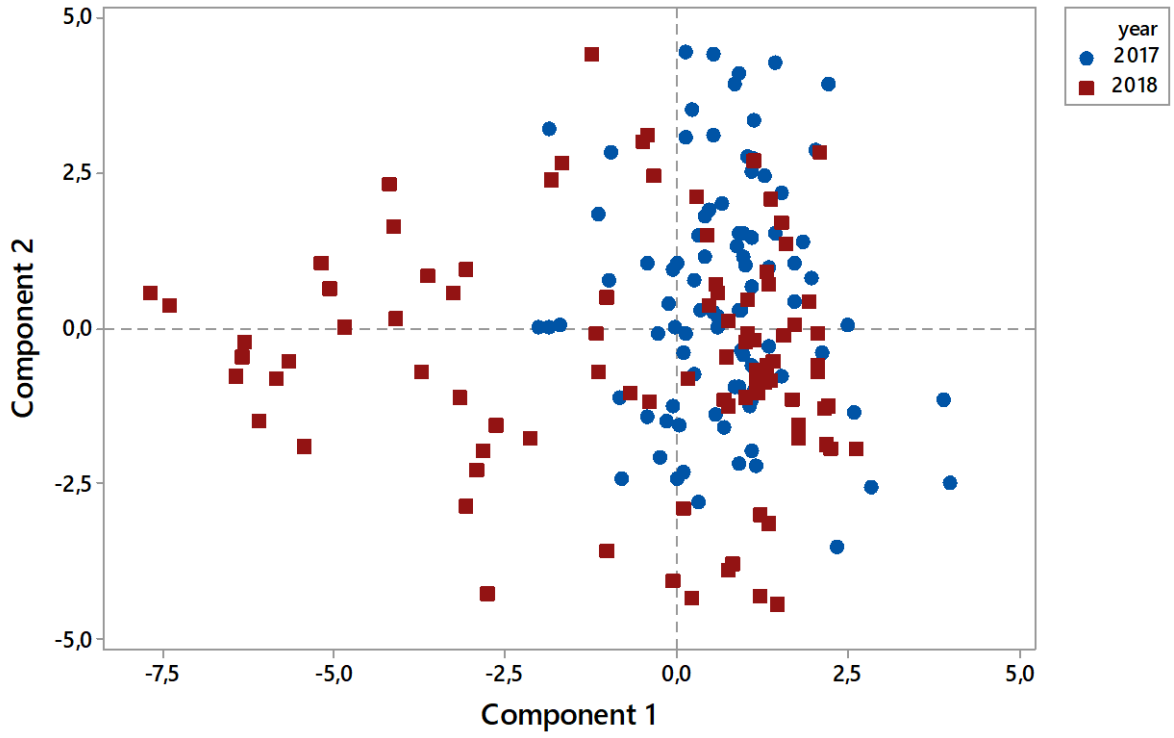


Figure 8: PCA showing the biplot distribution of the protein parameters along the first and second principal component for the years 2017 and 2018.

4.1 Wheat grain microstructure studied by LM

4.1.1 LM method establishment and optimization

The microstructure of wheat grain components such as protein and starch distribution, was studied with an aim to evaluate differences between the genotypes grown in contrasting climates. Different sample preparation complexities were encountered, as the first method gave no differences in visualization of the protein in the grain. This was the main reason to shift focus towards method optimization of sample preparation. The microstructure of a sliced wheat grain sample was studied using two ways: 1) no paraffin embedding (Figure 9A) and 2) with paraffin embedding (Figure 9B and C).The method including paraffin embedded sample, which was treated with 4% paraformaldehyde (PFA) and 2.5% glutaraldehyde (GA) solution (Figure 9C) gave the best result, compared to the direct grain staining (Figure 9A) or paraffin embedding and treatment with 4% PFA and 0.25% GA (Figure 9B). For further analyses, grain cross-sectioned sample, embedded into paraffin and stained for protein + starch, and further crosslinked with 4% PFA and 2.5% GA was used.

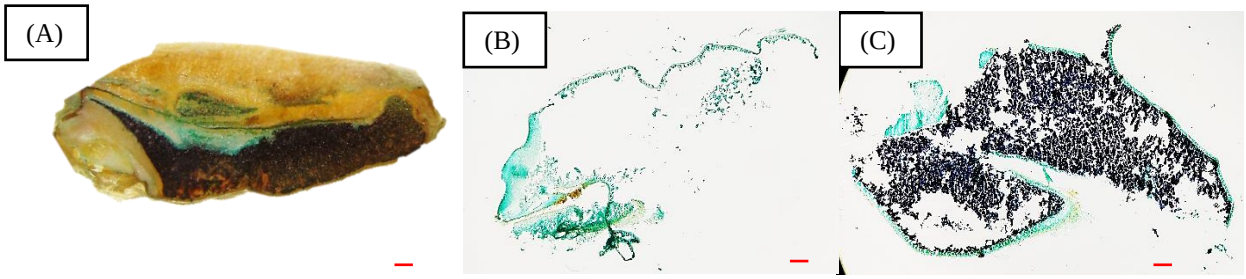


Figure 9: Microstructure of cross-sectioned wheat grain cultivars, (A) grain cross-sectioned and coloured for protein + starch; (B) paraffin embedded grain sample coloured for protein + starch and treated with 4% PFA and 0.25% GA solutions; (C) paraffin embedded grain sample coloured for protein + starch and treated with 4% PFA and 2.5% GA (A, B, C, 20x magnification and scale bar = 400µm).

4.1.2 Microstructure of selected genotypes from different years

Wheat grains of four genotypes, Sonett, 19, 21 and 28, grown in 2017 and 2018 were selected in order to investigate microstructure differences in the grains between the years. Two of the samples had a similar %UPP between the years 2017 and 2018 (19 and 28) and the other two had a large difference (Sonett and 21) (Table 7). The microstructures of the four selected spring wheat genotypes were compared (Figures 10-13). In general, the grain microstructure for all the

samples studied was rather fragile during the sectioning and washing steps, therefore it was difficult to obtain the entire structure of the cross-sectioned grain. Thus, no difference in protein and starch distribution could be identified. The starch microstructure seemed to be more dense and compact for two of the studied genotypes (Sonett and 19) from 2018 compared to 2017 (Figure 10 and Figure 11), while for the other genotypes (21 and 28) a more starch dense microstructure was found for the 2017 material compared to 2018 (Figure 12 and Figure 13). A stronger protein matrix that held the densely packed starch granules, seemed to appear for genotype 19 grown in 2018 (Figure 11D). This genotype was also among the ones that had the most stable gluten protein parameters, including %UPP observed by SE-HPLC. Overall, the microstructural analysis method should be further improved in order to get a better view of both protein and starch in the grain.

Table 7: Percentage of UPP of the selected lines for LM analysis.

Genotype	%UPP in 2017	%UPP in 2018
Sonett	29.96	46.65
19	50.64	52.92
21	43.41	57.79
28	46.12	46.76

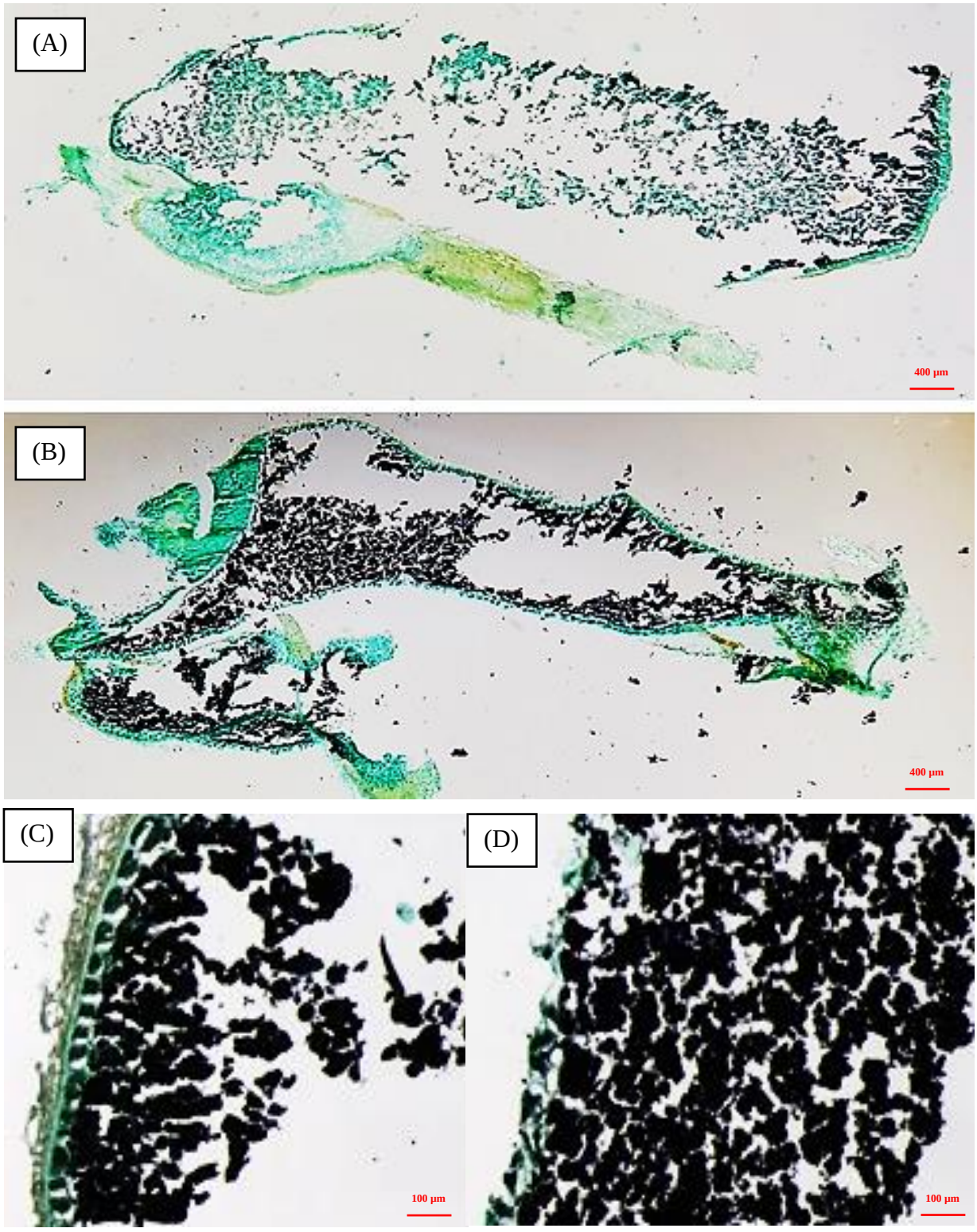


Figure 10: Whole grain section of Sonett grown in 2017 (A) and 2018 (B), (20x magnification and scale bar = 400µm). Closer-up view of whole grain section of cropped Sonett microstructure grown in 2017 (C) and 2018 (D), (100x magnification and scale bar = 100µm).

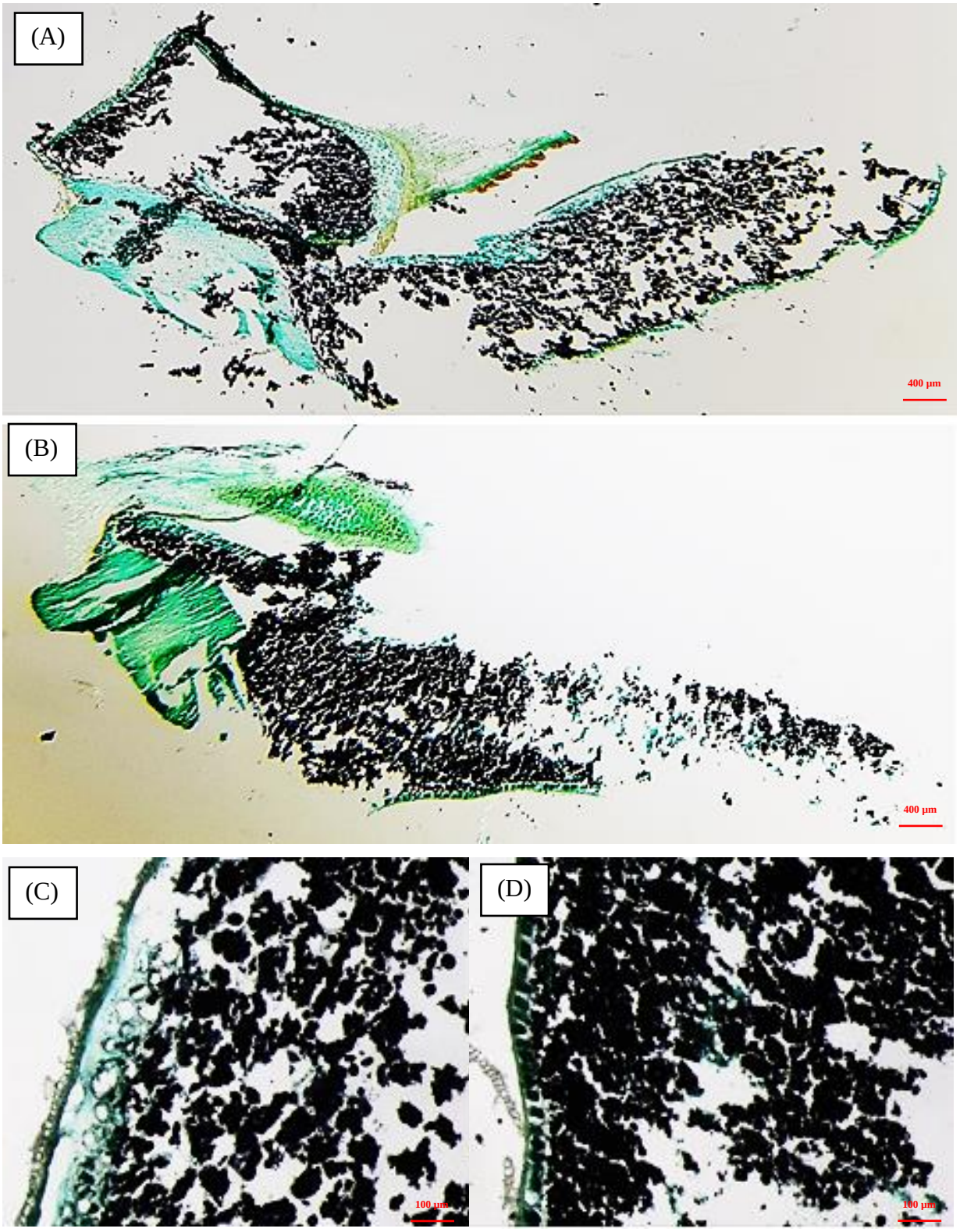


Figure 11: Whole grain section of genotype 19 grown in 2017 (A) and 2018 (B), (20x magnification and scale bar = 400µm). Closer-up view of whole grain section of cropped genotype 19 microstructure grown in 2017 (C) and 2018 (D), (100x magnification and scale bar = 100µm).

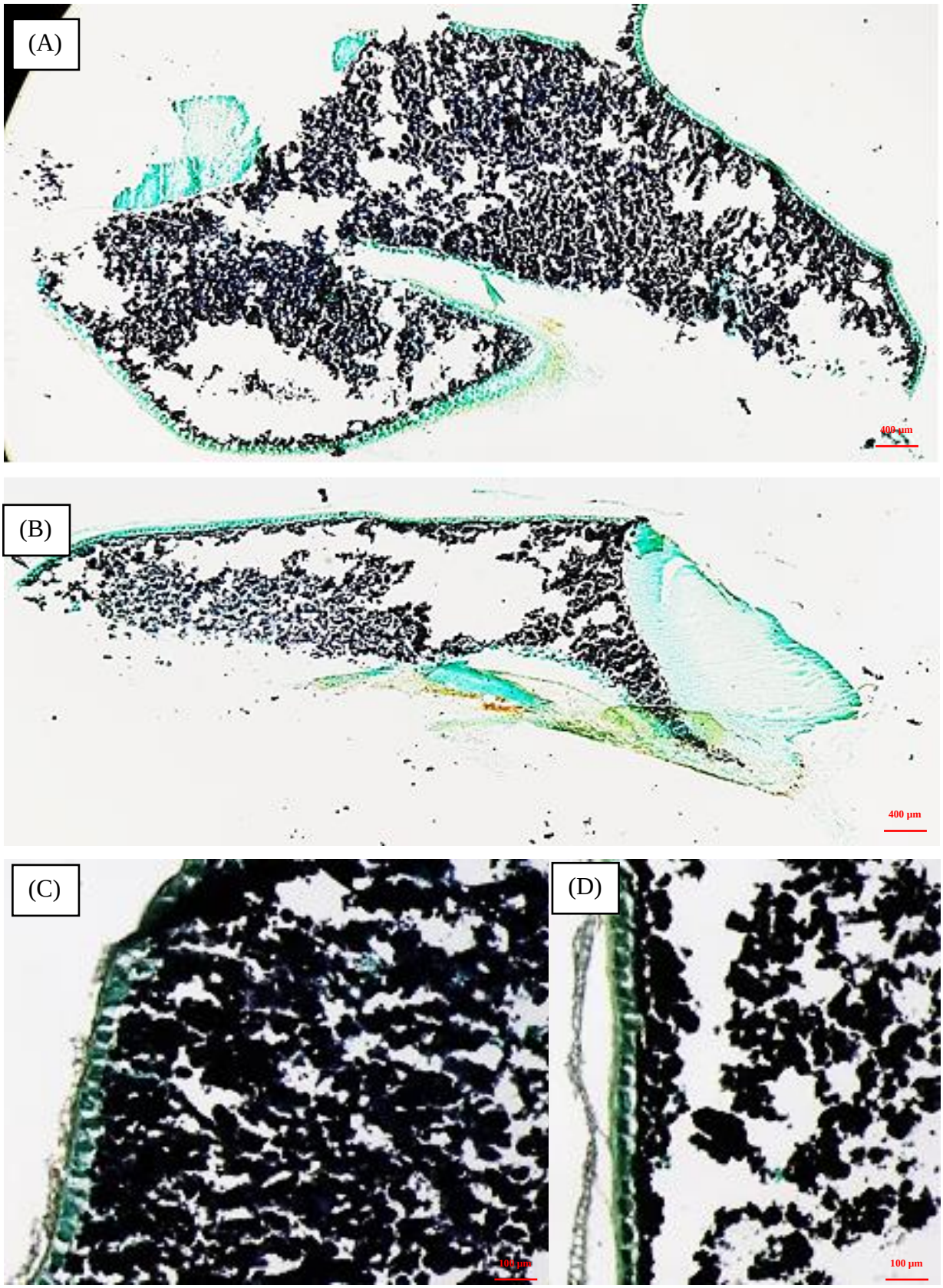


Figure 12: Whole grain section of genotype 21 grown in 2017 (A) and 2018 (B), (20x magnification and scale bar = 400µm). Closer-up view of whole grain section of cropped genotype 21 microstructure grown in 2017 (C) and 2018 (D), (100x magnification and scale bar = 100µm).

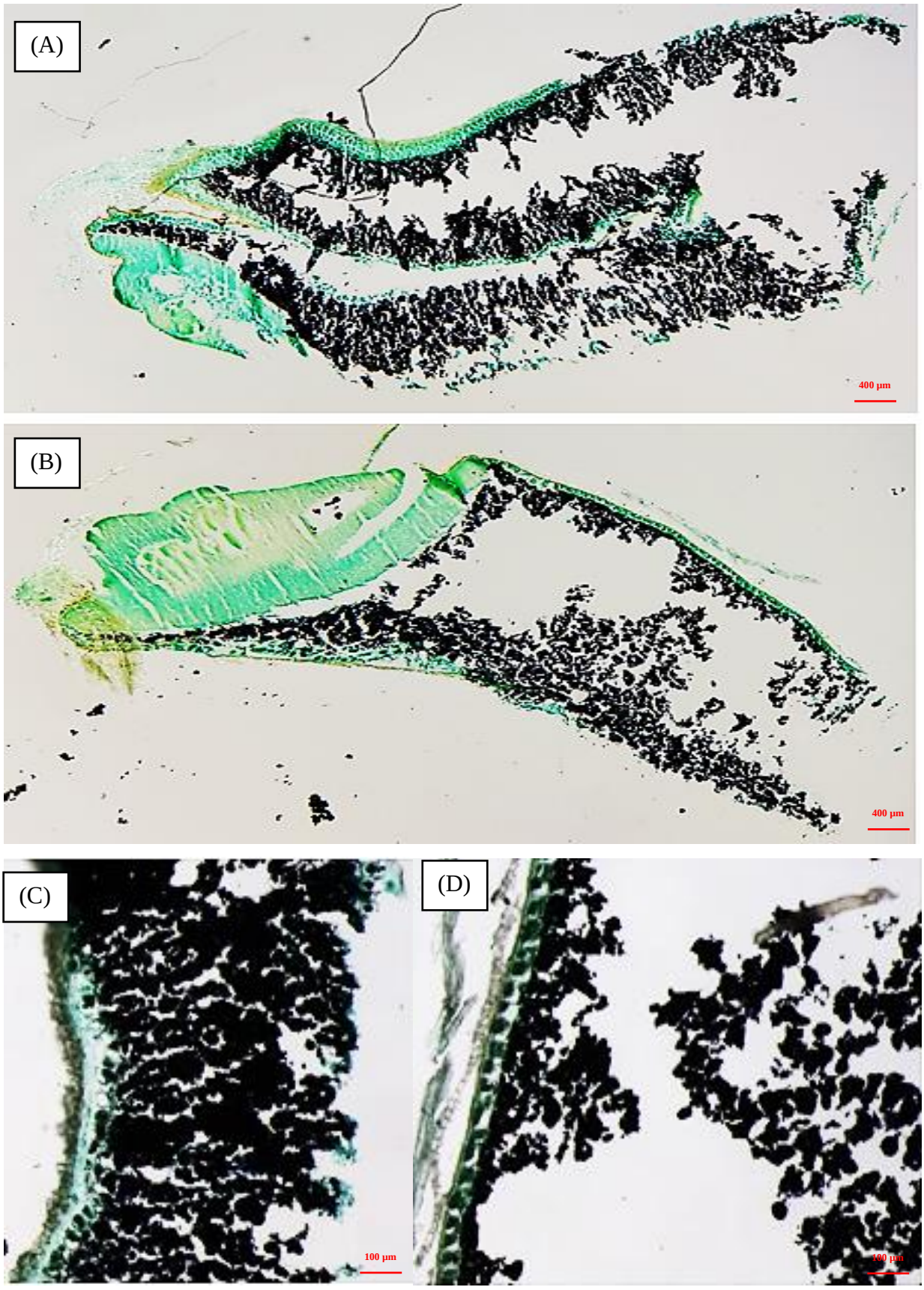


Figure 13: Whole grain section of genotype 28 grown in 2017 (A) and 2018 (B), (20x magnification and scale bar = 400µm). Closer-up view of whole grain section of cropped genotype 28 microstructure grown in 2017 (C) and 2018 (D), (100x magnification and scale bar = 100µm).

4. Discussion

The SE-HPLC results showed that the studied gluten protein parameters in wheat genotypes were affected differently by contrasting climate during 2017 and 2018. Drought and heat either induced, had no effect or reduced formation of the large gluten protein polymers and monomers, which have an impact on bread-making quality of wheat. Thus, this indicates that environmental conditions, such as temperature and precipitation, affect the amount and size distribution (composition) of gluten proteins and consequently also the bread-making quality characteristics (Johansson et al. 2005).

The growing season in 2017 was colder and with higher precipitation compared to the 2018 season, especially during the last months of the growing season, before the harvest. For the year 2018, it was much warmer, and nearly no rainfall in June, July and August compared to 2017, especially July 2018 was dry, getting only 4 mm rain. Thus, the cultivation period was considerably shorter in 2018 than 2017, which means that the wheat developed and matured faster in 2018 (Koga et al. 2015).

NIT results showed a higher protein % in 2017 for about $^{2}/_{3}$ of the genotypes compared to 2018 (Figure 4). SPP and LMP were mostly higher for 2017 compared to 2018 (Figure 5). LPP values showed many genotypes that differed greatly between the two years, and half of the genotypes were higher in 2017 compared to 2018. SMP were higher in 2018 and had many genotypes that differed greatly between the two years. For TOTE and TOTU, there were not that large differences between the years for most of the genotypes. It was rather %LUPP and %LUMP that had higher values during 2018, 22 out of 30 genotypes for %LUPP and 17 out of 30 genotypes for %LUMP. Higher temperature and a shorter growing period in 2018 decreased starch accumulation, increased protein concentration and the amount of polymeric proteins (Randall and Moss 1990; Johansson et al. 2013). Out of the 30 different wheat genotypes, there were four genotypes, Happy, 19, 22 and 28, that had %UPP higher than 40% and a smaller difference in %UPP (≤5%) between the two years. These genotypes were considered as the most stable genotypes. %UPP was used to select the stable genotypes because it is a well-known parameter associated with bread-making quality. For most samples, the values for %UPP in 2018 were higher than in 2017. It has been proven that there is a positive correlation between the amount of gluten protein and the proportion of %UPP, as well as temperature up to about 30°C in field grown wheat (Johansson et al. 2005). For the ratio of monomeric protein to polymeric protein,

there were not much of a difference between the genotypes for 2017 (Figure 7). However, there was a pronounced difference between the years for nine of the genotypes, as they had a much higher ratio in 2018 than in 2017. A higher value of the ratio monomers/polymers indicates more monomers compared to polymers.

The ANOVA result showed that the year (Y) had a significant effect on almost all of the protein parameters, except uLPP, uLMP and %LUMP. For genotype (G) and the interaction between genotype and year (GxY) there was a significant difference between all the measured parameters for all 30 genotypes. From this result, it can be concluded that G, Y and GxY had a large impact on different gluten protein (Malik et al. 2013).

The PCA result showed the distribution of protein parameters from 2017 and 2018 (Figure 8). The parameters of the material grown in 2018 were more scattered compared to the material from 2017. This means that there was a higher variation in the measured protein parameters in 2018.

Regarding the structural grain morphology, the preparation of sample slices needs to be improved, since the cutting of the paraffin embedded grains turned into powder. Only the first slices produced were intact. The fixative glutaraldehyde (GA) was used because it has a stronger fixation power compared to paraformaldehyde (PFA) (Heard et al. 2002; Ahmed et al. 2016; Van Herpen et al. 2008). Among the four different tested concentrations of PFA and GA, the highest concentration of PFA and GA provided the best results, 4% PFA + 2.5% GA. The concentration of PFA and GA in other studies vary from 2 to 4% and from 0.5 to 2.5%, respectively (Heard et al. 2002; Ahmed et al. 2016; Moore et al. 2016; Van Herpen et al. 2008). The reason why the chosen concentrations for the crosslinkers did not work well, could be that the fixation solution did not penetrate deep into the grains, and the slices that were produced were not completely intact, which induced a collapse of the sample. Thus, it was not possible to study the difference in protein and starch distribution in the grains between the two years. The only thing observed from the slices was that the selected genotypes from 2017 were a little more intact than the slices from 2018 (Figure 10-13). Further improvement of the paraffin fixation processing is needed. There is also a need to test other concentrations of PFA and GA in order to fixate the components of the grain. Maybe using a method called Hybrid-Cut tissue sectioning that is used for other plant tissue can help get better cuts (Chen 2016).

5. Conclusions

The contrasting climate, i.e. contrasting temperature and precipitation, affected the gluten proteins in wheat genotypes to varying degrees. Higher temperature and drought resulted in more pronounced differences in protein parameters among the genotypes, compared to the cooler climate with higher precipitation. However, some genotypes, i.e. Happy, 19, 22 and 28, produced similar values in %UPP over the two years. These genotypes might have the potential to withstand drought and heat and provide stable gluten strength that might result into stable bread quality. However, SE-HPLC analysis of these genotypes grown in several years and other bread-making quality tests (e.g. rheomix and baking) should be done in order to further test their bread-making stability.

For the light microscopy study, it was not possible to differentiate samples based on grain morphology. No differences could be identified in the protein and starch distribution in the grains between the two years among the studied genotypes, Sonett, 19, 21 and 28. Microstructure sections that were produced from the wheat grains embedded by paraffin were not equally intact for all the genotypes. Microstructure sections from the selected genotypes from 2017 were more intact compared with the slices from 2018. Further improvements are needed in order to develop the procedure of paraffin fixation of the grain to get the intact microstructure of the grain section.

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